Avery, MacLeod, and McCarty:

Revolutionaries or Puzzle-Solvers?

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The great names in the biology of the last hundred years are Darwin, Mendel, and Avery.
--Erwin Chargaff, Heraclitean Fire, (1978), p. 105.

Part of the lore of molecular biology is that the discovery which is its foundation went unrecognized for most of a decade. The discovery was that genes are made of DNA. It was announced in 1944, but according to the story, the profound biological significance of that discovery was not fully appreciated until the helical structure of DNA was discovered in 1953.

In the early 1970's when this story appeared in print, it was denounced by some scientists and historians as a fairy tale.[1] They pointed out that, in contrast to the case of Mendel, the discovery that the genetic specificity of living organisms was carried by molecules of DNA immediately commanded the attention it deserved.

Although it is certainly true that this discovery was not overlooked by the scientific community, it is also true that its full elaboration did not evolve until many years had passed. In this paper, I will describe the recognition that the 1944 results received; this will lend support to those who denounce the lore as a fairy tale. Nevertheless, I will not veil the limitations of that recognition. In the dispute about the origins of molecular biology, my intention is not simply to defend one side against the other; rather my intention is to explain why each of these opposing interpretations can claim some part of the truth.

(A) Introduction: Griffith's Surprise.

In February 1944 O.T. Avery, C.M. MacLeod and M. McCarty published a paper entitled "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types. Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III."[2] The phenomenon they were studying -- the transformation of pneumococcal types -- had been reported in January 1928 by an English pathologist, Fred Griffith.[3]

Pneumococci, the organisms which cause pneumonia, exist in a number of true-breeding types which can be serologically distinguished. If pneumococci of one type are grown in a medium containing type-specific antisera, their descendents will have lost their virulence, that is, their virulence will have become attenuated. There is a visual test for whether or not a strain has become attenuated: when grown on solid media, attenuated strains look rough (R); virulent strains look smooth (S). Thus any given type of pneumococci can come in an attenuated R form, and a virulent S form.

Griffith found that if very large amounts (50 ml.) of non-virulent (R) pneumococci were injected subcutaneously in mice, the mice died and S pneumococci (of the same type as that from which the injection was derived) could be recovered from the dead mouse. He supposed that the pneumococci which were injected had not completely lost the virulent S-antigen during attenuation, and he reasoned that when the organisms injected lyse they release this S-antigen which furnishes them with "a pabulum which the viable R pneumococci can utilize to build their rudimentary S structure."[4] Griffith further supposed that the mice survived injection of smaller quantities of the non-virulent R pneumococci; because only a small percentage of the R form of any type will retain the virulent S-antigen. He reasoned that if this explanation were correct, then he should be able to turn a small, non-lethal injection of R cells into a lethal injection by providing a non-lethal source of the S-antigen in the form of heat-killed S pneumococci. Griffith:

> It appeared possible that suitable conditions could be arranged if the mass culture was derived from killed virulent [S] pneumococci, while the living R culture was reduced to an amount which, unaided, was invariably infective. There would thus be provided a nidus and a high concentration of S antigen to serve as a stimulus or a food, as the case may be. 5

This hypothesis proved correct. The injection of dead (S) pneumococci of a given type in conjunction with living, attenuated (R) pneumococci of the same type proved lethal to the mice. And living, virulent (S) pneumococci -- again of that type -- could be recovered from the mouse.

Griffith's surprise was in his controls. To control his experiment, he injected living non-virulent R cells of one true-breeding pneumococcal type together with dead virulent S cells of a <u>different type</u>. He expected that the R organisms of one type would not be able to use the S antigen of a different type as a "stimulus or food as the case may be." He expected the mice to survive these particular injections. To his surprise the mice died, and he recovered from the dead mice living pneumococci not of the injected R type which had been alive, but of the injected S type which had not been alive.

As we look back on Griffith's surprise, its significance for molecular biology consists in its exhibiting the transformation of one genetically pure population into a different genetically pure population. But this could not have been what surprised Griffith.

In 1928, bacteria were not known to possess nucleoid bodies, and they were not thought about in genetical terms at all. R. Dubos, sometime collaborator of O.T. Avery, has written of the early response to the fluidity with which colonies of bacteria changed their characteristics: "In most cases, the diversity and complexity of the changes observed, the rapidity with which they occurred, and the ease of their reversibility made it difficult to believe that the chemical concepts of genetics sufficed to explain the variability of bacteria."[6] Griffith's paper itself exhibits this non-genetical approach to bacteria. To contemporary eyes the phenomenon of S to R attenuation is a clear illustration of natural selection operating on bacteria, but Griffith speaks about attenuation in Lamarckian and teleological terms which, today, have an odd sound:

> By assuming the R form the pneumococcus has admitted defeat, but has made such efforts as are possible to retain the potentiality to develop afresh into a virulent organism. The immune substances do not apparently continue to act on the pneumococcus after it has reached the R stage, and it is thus able to preserve remnants of its important S-antigens and with them the capacity to revert to the virulent form. While the R form may be the final stage in the struggle of the bacterium to preserve its individuality, I look upon the occurance of the various serological races as evidence of similar efforts to contend against adverse circumstances. 7

In 1928, scientists were not thinking about bacteria genetically but they were thinking about them in terms of types. Griffith's result was surprising because it suggested that the widespread belief that there were immunologically specific, true-breeding types of pneumococci was mistaken. Coincidentally, Avery had played a major role in establishing this belief; thus R.D. Hotchkiss comments:

> Small wonder that [to Avery] the work of Griffith in [1928]...seemed doubtful and contrary to all that had been carefully established; for this young English microbiologist described transformations that seemed to be conversions of one true-breeding type into another.[8]

(B) The 1944 Paper.

Soon after Griffith published his surprising result, Avery's laboratory at the Rockefeller Institute in New York City attempted first to reproduce and then to extend Griffith's work. The history of these early investigations has been told by R. Dubos and, in even more detail, by M. McCarty in his recently published book, The Transforming Principle.[9] I will pick up the story after Alloway, in 1932, had isolated a cell-free extract which was capable of inducing transformation of pneumococcal types.[10]

At this point studies on the chemical nature of the substance inducing transformation could begin. Such studies were undertaken at the Rockefeller by Avery, first in collaboration with C. M. MacLeod and then, after 1941 (when MacLeod moved from the Rockefeller to NYU and McCarty moved from NYU to the Rockefeller), in collaboration with M. McCarty. With one curious three year hiatus from 1938 to 1940, these joint studies were pursed continuously from 1934 to 1943, but Avery's lab did not publish anything on transformation during this period.[11] The chemical identity of the transforming principle proved especially difficult to discern. After all the hard work of its chemical analysis had been accomplished, Avery wrote to his brother Roy in May of 1943:

> The crude extract (Type III) is full of capsular polysaccharide, C (somatic) carbohydrate, nucleoproteins, free nucleic acids of both the yeast [RNA] and thymus [DNA] type, lipids and other cell constituents. Try to find in that complex mixture the active principle!! 12

Of course this retrospective account makes the process of chemical analysis sound too easy; because Avery and his co-workers began by knowing nothing of what was in Alloway's cell-free extract. Alloway described it as a "thick syrupy precipitate."[13]

Avery, MacLeod, and McCarty faced three problems: (1) to purify a substance X from this syrup which was still capable of inducing transformation, (2) to identify the chemical nature of this X, and (3) to give a biological explanation of the transformation phenomenon.

The solution to (1) was rather difficult to find. The method of purifying X and demonstrating that it retained the ability to transform pneumococcal types proved to be very delicate. According to McCarty the laboratory notes from as late as 1940 and 1941 are "...sprinkled with the description of experiments that failed because 'the system was off.' [And he comments:] Sometimes this was due to a slip-up in the handling of one of the known components, but more often the responsible variable was never identified."[14] Because the full story of the solution to (1) is eloquently recounted in McCarty's book, I will not discuss it further. Exciting as it was it was only the discovery of a (usually) reliable procedure for isolating a biologically active precipitate from the thick syrup obtained by Alloway.

In the 1944 paper, Avery, MacLeod, and McCarty argued that the answer to (2) was that X was DNA. These arguments, presented in a section called "Analysis of Purified Transforming Material," were the heart of their paper. They presented seven considerations which together suggested that X was very pure DNA. I summarize those four considerations which were most relevant to the controversy which followed publication.[15]

a. The transforming substance X exhibited "little or no serological reactivity" with antisera to the pneumococci type from which it was derived. This suggested that X was not the sugar capsule of the S pneumococcus or any part of its antigen.[16]

b. They found that the Millon test, a qualitative test for protein, was negative on X. The Dische reaction for DNA was strongly positive. The Bial test for RNA was "weakly positive," but so was that for other preparations known to be DNA. These results suggested that X was more likely to be a nucleic acid than a protein.[17]

c. Various enzymes were tested for their ability to inhibit transformation, i.e. their ability to digest X. In 1944, no pure DNase was available; but all those enzymes capable of inhibiting transformation (and only those) were also able to digest authentic samples of

DNA. These results suggested that of the nucleic acids, X was more likely to be DNA than RNA.[18]

d. The transforming substance was very active in their transformation system: 0.003 micrograms were capable of inducing transformation at a concentration of one part in 600,000,000. Thus it seemed unlikely that a trace impurity was the source of X's biological activity. X seemed to be pure DNA.[19]

This was the core of Avery, MacLeod and McCarty's evidence that X = DNA. This was there answer to problem (2), the chemical nature of the transforming material.

The authors of the 1944 paper did not explicitly endorse any solution to problem (3); they did not explicitly offer any biological explanation of the phenomenon of transformation. In the discussion section of the 1944 paper, the authors merely listed four possible hypotheses about "The Nature of the Changes Induced" by the DNA fraction without deciding between them, and apparently without even suggesting which hypothesis the authors favored. The four hypotheses were due to Griffith, Dobzhansky, Stanley, and Murphy.[20]. The authors did not decide which of these four hypotheses was the most plausible; the conclusion of the 1944 paper said simply and safely:

> The evidence presented supports the belief that a nucleic acid of the desoxyribose type is the fundamental unit of the transforming principle of the Pneumococcus Type III.[21]

(C) The Lure of the Lore

I have said that it is part of the lore of molecular biology that the genetic significance of the 1944 results was not fully appreciated until the early 1950's. As the story goes, this delay was primarily the result of two hesitancies in the 1944 paper: (a) the hesitancy with which Avery, Macleod and McCarty reported their discovery that the transforming principle was DNA and (b) the hesitancy with which they associated DNA with the genetic material of the Pneumococcus. These supposed hesitancies may be thought of as the lure of the lore.

It is sometimes thought that the great influence of Avery's discovery is inconsistent with the claim that the 1944 paper was, in these ways, hesitant. That is not true; the question of hesitancy and the question of influence are two separate questions: one concerns a printed text, the other concerns the response to that text.

In this section I try to demonstrate that the 1944 text was hesitant in both the ways I have mentioned, but I will also argue that neither of these hesitancies is sufficient to account for the delay between the discovery of the chemical nature of the gene and the explosion of molecular genetics in the 1950's. The lure is real, but it should be resisted.

(a) X = DNA.

In the discussion section of the 1944 paper we can find the sentence: "If it is ultimately proved beyond a reasonable doubt that the transforming activity of the material described is actually an inherent property of the nucleic acid, one must still account on a chemical basis for the biological specificity of its action."[22] This sentence suggests that, in 1944, the authors did not think they had proved, beyond a reasonable doubt, that X = DNA. Wendell Stanley, whose hypothesis that transformation was a viral phenomenon was mentioned in the 1944 paper, remarked in 1970 that the way the paper was written "...did not tend to imbue the reader with confidence in their results."[23]

It is probably fair to attribute this hesitancy to Avery; because during his summer vacation in Maine, he roughed out the introduction and discussion sections of the paper.[24] That this should have had a restraining effect on the interpretation offered in that paper might be guessed from a passage in a letter Avery sent to his brother Roy in May 1943: "It's hazardous to go off half cocked -- and embarassing to have to retract later."[25] MacLeod recalls that Avery was "...almost neurotic about overstating the case" for their analysis of the chemical nature of the transforming material. [26]

Dubos has offered an intriguing psychological explanation for what he also refers to as Avery's "...restraint and self-criticism bordering on the neurotic...."[27] In 1916 and again in 1917 Avery had published some premature specualtions which were amply refuted soon after publication. It is true that these early mistakes were embarrassing to Avery, and it is true that at the end of his scientific carreer Avery was more hesitant about taking risks in print than MacLeod thought reasonable. But it is difficult to know how to evaluate Dubos' suggestion that these two features of Avery's career were related as cause to effect. Dubos may have discovered a psychological explanation for why the 1944 paper only tentatively asserted that X = DNA; however, even if that explanation is rejected, we can still ask: does the tentativeness with which the 1944 paper claimed that X was DNA explain why the genetic significance of the 1944 results went unrecognized for ten years? The answer, I think, is no.

The simplest defense of this claim is that that early hesitancy was short lived. In 1946, McCarty and Avery extended their evidence, in two papers presented as Parts II and III of their 1944 "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types."[28] A pure preparation of DNase, which only depolymerized DNA, would have been a valuable contribution to the 1944 analysis, but at that time the only enzymatic systems containing DNase were impure; they contained other enzymes not specific to DNA or even to nucleic acids. It is against this background that McCarty and Avery reported in 1946:

> Since no purified preparation of desoxyribonuclease was available, purification of the enzyme was undertaken in this laboratory in order that enzymatic evidence concerning the nature of the transforming substance could be made more direct and conclusive. 29

The DNase they prepared did not give entirely unambiguous support to the claim that X = DNA; because it did exhibit some proteolytic activity. Nevertheless, as McCarty and Avery point out:

> The results of the present investigation show that in order to detect proteolytic activity, it is necessary to use an amount of purified desoxyribonuclease 100,000 times greater than that required to cause rapid and complete destruction of activity of the transforming substance. 30

Thus they concluded that there was "little doubt" that X = DNA.[32]

If the scientific community harbored any doubts about the conclusiveness of the 1944 evidence, the improved evidence of 1946 might have been expected to answer them. But it did not. Part of the reason may have been that, as McCarty cautiously puts it, "there is some evidence to suggest...that papers II and III (which finally appeared in February 1946, just two years after paper I) were not very widely read."[32] However even those who read them were not entirely convinced. Among these was A. E. Mirsky who, like Avery, worked at the Rockefeller Institute. Mirsky refused to be convinced that X was pure DNA, and McCarty notes that "since he [Mirsky] was widely acquainted with biologists and biochemists, [his]...dim view of the implications of our work certainly reached many ears and undoubtedly had some influence on its reception."[33] Why did Mirsky's respond in this way?

During the period I am discussing -- 1944 to 1953 -- Mirsky's own research was concerned with what was called chromosin, a DNA-protein complex found in cell nuclei, and he favored the hypothesis that X was just such a DNA-protein complex. The lengths to which he went to defend the viability of this hypothesis reached almost as far as a general inductive skepticism. In 1947 he claimed that the enzymatic evidence was not adequate to discriminate the possibility that the transforming principle was DNA from the possibility that it was a DNA-protein complex.[34] In 1950 Mirsky almost reached general skepticism when he claimed that "it is difficult to eliminate the possibility that minute quantities of protein that probably remain attached to DNA, though undetectable by the tests applied, are necessary [even if not sufficient] for activity...."[35] As

McCarty implies, Mirsky's stubborn refusal to let the evidence convince him was significant because his position as an expert on DNA chemistry made his doubts seem more than merely stubborn. (It also exemplifies the philosophical truth that there is no criterion for when reasonable doubts become unreasonable.)

It may come as a surprise to note that Mirsky's objections to the claim that X = DNA actually made the genetic significance of transformation more obvious not less obvious. In 1947, the distinguished geneticist H.J. Muller published a genetic interpretation of transformation which, at least in part, was based on Mirsky's stubborn objections to the views of Avery, MacLeod and McCarty. Muller thought that X was a gene; because he thought that X was not DNA. Muller writes:

> ...on 28 January [1946], Mirsky gave reasons for inferring that in the *Pneumococcus* case the extracted "transforming agent" may *really have had* its *genetic proteins* still *tightly bound to the polymerized nucleic acid*; that is, there were, in effect, still viable bacterial "chromosomes" or parts of chromosomes floating free in the medium used. These might, in my opinion, have penetrated the capsuleless [rough] bacteria and in part at least taken root there, perhaps after having undergone a kind of crossing over with the chromosomes of the host. 36 (my emphasis)

Thus, Mirsky's view of the chemical nature of X was actually part of Muller's justification for claiming that X, whatever it was, was the nearest anyone had come to putting a gene in a tube.

An explanation for this effect of Mirsky's objections and indeed of Mirsky's objections themselves is at hand. The chemical structure of nucleic acids was thought to be far too repetitive to carry biological specificity. The reason was that it was widely believed that the structure of nucleic acids was ABCDABCDABCD where the four letters stand for the four nucleotides. This was called the tetranucleotide hypothesis. As Delbruck puts it, "...it was believed that DNA was a stupid substance, a tetranucleotide which couldn't do anything specific."[37] Such a repetitive structure, it was felt, could play no more than a stabilizing role in the biological events of cell division: perhaps the chromosomes consisted of a backbone of nucleic acid around which the genetically active proteins were wrapped. Surprisingly, for this very reason, the 1944 result might have encouraged geneticists to attempt to isolate the supposedly genetically active protein component as part of an attempt to understand the biochemical activity of genes, but it did not.

Summary. Avery, MacLeod and McCarty's reservations about asserting X = DNA, cannot adequately explain the lack of attention paid to the genetic significance of transformation in the late 1940's and early 1950's. There are two probable reasons for this. First, in 1946 new enzymatic evidence should have quelled serious doubt about the nature of X. Second, even if, following Mirsky, those enzymatic results were not believed this might have made X look more like a gene, not less. Whether the new enzymatic results of 1946 were taken as conclusive or not, this gene in a tube seems not to have sparked the genetic interest it warranted.

(b) X = Gene

It is difficult to find an unequivocal biological explanation of the transformation phenomenon in the 1944 paper. The reason for this is probably that Avery was not as interested in how X functioned as he was interested in what X was. In his May 1943 letter to his brother Roy, after indicating that, like a gene, X was hetero- and auto-catalytic, Avery commented.

> Sounds like a virus -- maybe a gene. But with mechanisms I am not now concerned -- One step at a time -- and the first is, what is the chemical nature of the transforming principle? Someone else can work out the rest. Of course, the problem bristles with implications. It touches the biochemistry of the thymus type of nucleic acids [DNA] which are known to constitute the major part of the chromosomes but which have been thought to be alike regardless of origin and species. It touches genetics, enzyme chemistry, cell metabolism and carbohydrate synthesis, etc. [But] today it takes a lot of well-documented evidence to convince anyone that the salt of desoxyribose nucleic acid, protein-free, could possibly be endowed with such biologically active and specific properties and this evidence we are now trying to get. It's lots of fun to blow bubbles -- but wiser to prick them yourself before someone else tries to. So there's the story Roy....Talk it over with Goodpasture but don't shout it around -- until we are quite sure or at least as sure as present method permits. It's hazardous to go off half cocked -- and embarassing to have to retract later. 38

This well-known passage indicates that, as late as three months before the 1944 paper was written, Avery was not fully satisfied that X was pure DNA. As I have already argued, this should have been clear from the final, published version. Indeed, McCarty reports that even after that publication, in 1945, Avery "...continued to be plagued by nagging doubts about whether we were right...."[39]

More significantly the letter indicates that Avery was not concerned to define the biological mechanism at work during transformation. He was sure that the problem of transformation bristled with biological significance, but he did not feel inclined to work out the precise nature of this significance. "Someone else can work out the rest." This may explain why, in the discussion section of the 1944 paper, the authors merely listed four possible hypotheses without deciding between them. The four hypotheses were: (i) Griffith's: assimilating X to a stimulus or pabulum required for the manufacture of the S antigen; (ii) Dobzhansky's: assimilating transformation to the induction of a genetic mutation; (iii) Stanley's: assimilating transformation to infection by a virus; and (iv) Murphy's: assimilating transformation to the stimulation of tumors in healthy chickens by the injection of cell-free extract from tumors in other chickens.[40] The authors did not decide which of these four hypotheses was the most plausible because, as Avery wrote to his brother, that wasn't their primary concern and because, as they wrote in the paper: "In the present state of knowledge any interpretation of the mechanism involved in transformation would be purely theoretical."[41]

This last sentence, which represents a refusal to become involved in theoretical interpretations of their solid experimental results, was apparently typical of Avery's approach to science. Dubos remembers that "...Avery questioned the validity of biological generalizations and was even reluctant to use the word gene. He was virtually ignored by the theoreticians of genetics, precisely because he made no effort to communicate with them...."[42] Theoretical biology was not to appear above Avery's name as it might above Delbruck's and Crick's -- not for him, the Popperian conjecture, bold and improbable.

My next task is to determine whether this second hesitancy of the 1944 paper might have been the cause of what McCarty refers to as "...the apparently rather restrained acceptance of the thesis advanced in the 1944 paper."[43] One might reason: if the 1944 paper only hesitantly claimed that X = gene, then one can explain why the full recognition of the genetic significance of Avery's work was retarded: Avery's conservatism was the cause of this retarding. Nevertheless, I do not think this explanation is entirely persuasive and for three principal reasons.

(1) One important consideration is that the same 1946 papers that made up for the originally hesitant claim that X = DNA also made up for the originally hesitant claim that X = gene. Thus the factual basis for this explanation of the delay is not secure. I will now briefly recount the evolution of the Rockefeller group's interpretation of bacterial transformation.

In his May 1943 letter to his brother Roy, Avery discussed the genetic significance of his investigations of transformation:

> If we are proven right, and of course that's not yet proven, then it means...that by means of a known chemical substance it is possible to induce predictable and hereditary changes in cells. This is something that has long been the dream of geneticists. The mutations they induce by X ray and ultraviolet light are always unpredictable, random and chance changes. If we are proven right -- and of course that's a big *if* -- then it means that both the chemical nature of the inducing stimulus is known and the chemical structure of the substance produced is also known.... 44

Nestled among his cautionary disclaimers is Avery's belief that they had realized what he called the dream and what Muller called the "Eldorado of geneticists": directed mutation.[45]

Not long after writing this letter, Avery went to Maine for the summer. As I have already observed, while he was there he wrote the first drafts of the opening and closing sections of the 1944 paper which was submitted for publication on November 1, 1943. It is not therefore surprising that some key words of Avery's letter reappeared in the first sentences of that article.

These sentences also represent the closest that paper came to giving a genetic interpretation of transformation:

> Biologists have long attempted by chemical means to induce in higher organisms predictable, and specific changes which can thereafter be transmitted in series as hereditary characters. Among microorganisms the most striking example of inheritable and specific alterations in cell structure and function that can be experimentally induced and are reproducible under well defined and adequately controlled conditions is the transformation of specific types of Pneumococcus. 46

If we recall that Dobzhansky is quoted in that paper as believing pneumococcal transformations to be "...authentic cases of induction of specific mutations by specific treatments,"[47] then the thought occurs that in spite of their claims to the contrary, the authors of the 1944 paper were not entirely agnostic about the biological interpretation of transformation. Without being explicit, they seem to have slipped an endorsement of Dobzhansky's interpretation between the lines of their paper.

If I am right so to interpret the 1944 paper, then it becomes clear that, in 1946, McCarty and Avery changed their explanation of the biological mechanisms underlying transformation. The 1946 evidence was inconsistent with the hypothesis that transformation was directed mutation: DNA fractions from type III R cocci are indistinguishable from type III S DNA fractions except that the former is not active in the transforming system and the later is. If DNA were a mutagen then these results would not be expected. A different interpretation is suggested in 1946; to my mind it identifies DNA as carrying the genetic specificity of bacteria, but curiously, it refrains from saying so explicitly. Here is a full fledged genetic interpretation which refuses to use the word "genetic":

> It is possible that the nucleic acid of the R pneumococcus is concerned with innumerable other functions of the bacterial cell, in a way similar to that in which capsular development is controlled by the transforming substance. The desoxyribonucleic acid from type III pneumococci would then necessarily comprise not only molecules endowed with transforming activity, but in addition, a variety of others which determine the structure and metabolic activities possessed in common by both the encapsulated (S) and unencapsulated (R) forms. 48

Thus, although the 1944 paper did indeed refrain from identifying the transforming principle with the genetic material, this hesitancy was gone by 1946. The hesitancy of the first paper is therefore unable to explain the tepid reception given 1944 paper by geneticists.

(2) It could be argued that even the 1946 paper was too hesitant to have envigorated genetic interest in bacterial transformation. However McCarty and Avery were not the only scientists publishing genetic interpretations of transformation and other scientists were not so circumspect as to avoid the word "gene". In 1947, A. Boivin published a paper read at a Cold Spring Harbor Symposium of the same year. Holding nothing back, Boivin called the paper "Directed Mutation in Colon Bacilli, by an Inducing Principle of Desoxyribonucleic Nature: Its Meaning for the General Biochemistry of Heredity." And what was its meaning for the biochemistry of heredity? Boivin:

> In bacteria -- and, in all likelihood, in higher organisms as well -- each gene has as its specific constituent not a protein but a particular desoxyribonucleic acid which, at least under certain conditions (directed mutations of bacteria), is capable of functioning <u>alone</u> as the carrier of hereditary character; therefore, in the last analysis, each gene can be traced back to a macromolecule of a special desoxyribonucleic acid...This is a point of view which, in respect to the actual state of biochemistry, appears to be frankly revolutionary. 49

Unlike McCarty and Avery, Boivin does not seem to have discriminated the posssibility that DNA was directing a mutation from the possibility that it was the carrier of hereditary character, but in this passage he offers an extremely explicit account of the genetic significance of transformation. Thus by 1947, there had been published a remarkably theoretical paper which asserted that genes were made of DNA with none of the hesitancy concerning the word "gene" which characterized McCarty and Avery's 1946 publications. Furthermore (although it is only marginally relevant, since I am here surveying published reports), we have it on McCarty's authority that the quotation from Boivin above is "...certainly a fine statement of what we believed but were too reticent to say."[50]

It must be said that Boivin's paper was no more effective than the papers of Avery and his colleagus at envigorating genetic investigations of transformation or the transforming principle. Part of the reason may have been that Boivin's theoretical approach to genetics was at the time of this publication, rather idiosyncratic. By 1960 it would not have been.

(3) It is sometimes felt that both Avery's and Boivin's genetic interpretations of transformation were unlikely to affect practicing geneticists; because they weren't given by card-carrying geneticists. However, this argument is inadequate as an explanation for why the Avery results were not more vigorously pursued; because at least three geneticists promptly offered genetic interpretations of the phenomenon. I have already mentioned Dobzhansky's which was published in 1941 and Muller's which was published in 1947. Sewell Wright, the

distinguished biochemical and population geneticist, may be added to the list, for in 1945 he wrote of pneumoccocal transformation; "The results suggest chemical isolation and transfer of a gene rather than induction of mutation."[51] This is a succinct and brilliantly clear statement.

Summary. The undeniable hesitancy of the 1944 paper with respect to the genetic mechanisms underlying pneumococcal transformation is unable to explain why this paper was not more vigorously pursued in the decade following its publication. There are three reasons for this. In 1946, McCarty and Avery did publish an interpretation of transformation which is genetic in everything but name. In 1947, Boivin followed McCarty and Avery but added the name. Finally, the genetic interpretation was also subscribed to -- in print -- by card-carying geneticists.

In this section I have supported the traditional view that the 1944 paper did not unequivocally assert either that X = DNA or that X = gene. Nevertheless I have rejected the traditional view that these two hesitancies were the cause of the delay between Avery's announcement and the explosion of molecular genetics.

(D) Four Ways the Avery Result was Pursued.

The discussion of the last section raises an issue which puts the accepted lore of molecular biology in question. Is it true that the Avery result was only tentatively accepted?

In recent discussions, this question has received conflicting answers. On the one hand an examination of the number of times the 1944 and 1946 papers were cited reveals that they were not frequently given as references in papers by those concerned with the genetics of microbes.[52] This suggests that Avery's papers were not considered very important. On the other hand, it has been suggested that their importance was so obvious that they would not have required citation any more than Mendel's papers would have required citation.[53]

If I had to choose sides in this debate I would unhesitatingly report that the Avery result was widely known even if it was not widely referred to. But I would rather not choose sides at all; because I think this particular debate masks what is really interesting about this period of scientific development.

The issue is not whether or not scientists knew about the 1944 paper. They did. Although The Journal of Experimental Medicine had a "...fairly limited readership,"[54] the Avery result was never lost to the scientific community in the way Mendel's work was. What I want to discuss is not simply whether or not the Avery result was known, but whether or not its profound implications were fully comprehended. I am struck by the fact that this failure to comprehend is even acknowledged in R. Olby's attack on the lore of Avery's non-recognition. Olby: "Only with the advantage of hindsight can we see the significance of the 1944 paper as obvious."[55] The qestions are thus raised. What gives us this advantage of hindsight? And what is it about the way Avery's result was pursued that reveals its significance not to have been fully grasped? Avery's work was not universally ignored, but we must determine the particular ways it was acknowledged. It was not immediately developed in the directions which, with hindsight, appear obvious. Why?

It hardly needs pointing out that what gives us the advantage of hindsight is that we know of the explosion of what might be called theoretical genetics which followed the publication of the April 25, 1953 issue of Nature. In that issue, over the names J.D. Watson and F.H.C. Crick, there appeared an article titled "Molecular Structure of Nucleic Acids. A Structure for Deoxyribose Nucleic Acid." Hindsight gives us this advantage: Avery's work initiated the biochemical investigation of genetic phenomena which provided all of the most exciting work in molecular biology for more than a decade following 1953. With significant exceptions, what seems not to have been recognized by those who pursued the Avery results between '44 and '53, is that those results called for chemical investigation with an eye to the way in which information was encoded in DNA.

I should say that investigators at the time may have thought that this was the direction in which the Avery result was pointing. But these same investigators might either not have known how to, or not cared to, move their research off in that direction. Whatever the explanation should be, it is a fact that chemical investigations were <u>not</u> an immediate consequence of the 1944 publication.

Hotchkiss
Chargaff

We may distinguish four different ways in which biologists acknowledged the Avery result. Rather arbitrarily, I will associate them with the names of one of the major contibutors to each of these four ways of pursuing the results of Avery, MacLeod, and McCarty: (i) in the manner of R.D. Hotchkiss, (ii) in the manner of J. Lederberg, (iii) in the manner of M. Delbruck, and (iv) in the manner of E. Chargaff. I hope to show that only the last grants Avery the position granted him by hindsight.

(i) In July 1946, McCarty left Avery's pneumococcal research team to take command of the Rockefeller's streptococcal laboratories.[56] In his memoirs, McCarty asks himself a question that hindsight makes unavoidable: "how could one even consider turning from the path of research opened up by the DNA discovery"? His reply is that, perhaps because he was not trained as a geneticist, he was "...little attracted to pursuing the studies along genetic lines." His own inclination would have been to attempt to purify from the active DNA fraction that component whose sole function was to induce synthesis of the type-specific capsular polysaccharide. For my purposes, what is worth noting in McCarty's memoirs is that if he had continued working on transformation he would not have left the field of questions opened up by Griffith and pondered by Avery.

Already in 1938, before McCarty arrived at the Rockefeller, Hotchkiss had asked Avery whether he might investigate some of the questions raised by the phenomenon of transformation; Avery said: not now. Finally as McCarty was leaving the pneumococcus, Hotchkiss was permitted to participate. Hotchkiss represents the line of research closest in spirit -- and in body -- to Avery himself and farthest in spirit from what hindsight discloses as the natural path of research. His concern was to demonstrate the general genetic significance of the Avery result by showing that DNA was able to induce transformation of characteristics other than capsular synthesis.[57] In this endeavor Hotchkiss was successful. Hotchkiss also attempted to improve the evidence that transformation by the DNA function was not being accomplished by proteins contaminating that fraction. This again was successful.[58] What distinguishes Hotchkiss' manner of pursuing the DNA discovery is the way in which Avery's own questions and answers guided his research. Hotchkiss' bacteriological investigations though elegant and original are not on the path that, in hindsight, stretches between Avery, and Watson and Crick.

(ii) J. Lederberg has argued powerfully that although the Avery result suggested genes were bits of DNA, the evidence "...was not yet conclusive for it was still controversial whether bacteria could even be thought of as having a genetics."[59] Lederberg and his mentor F.J. Ryan therefore tried to produce the transformation phenomenon in a non-bacterial system using the red bread mold Neurospora. (Note the modulation required to give significance to what we call the "repetition" of an experimental result.) Their efforts were not successful, and to this day, Neurospora have never been shown to exhibit transformation.

As Lederberg describes it, the work he did with E. Tatum at Yale (and for which, in 1958, he received, with Tatum and Beadle, a Nobel prize) was a direct result of those null results with Neurospora. Lederberg: "One day I suggested that we [i.e. F.J. Ryan and himself] ring the changes on our experimental approach. Instead of trying to make Neurospora imitate a phenomenon recently worked out in bacteria, we could use similar methods to inquire whether bacteria had genetic mechanisms similar to Neurospora."[60] Without wishing in any way to deny the brilliance of the resulting discovery and investigation of microbial genetics, this research is not part of what (in hindsight) appears as the new wave, namely, chemical investigation of the gene. Lederberg's line of research demonstrated that the principles of Mendelian genetics -- thoroughly investigated in the fruit fly -- could also be applied to bacterial systems. As Lederberg put it in 1947, "...since we have been able to demonstrate no appreciable point of difference between the features of gene exchange in this strain of E. coli and in the classical materials of Mendelian experimentation, the most economical conclusion is that the mechanisms involved are also similar."[61] Lederberg's investigations were not, any more than Hotchkiss', in the biochemical lineage that ties Avery's work to Watson and Crick.

(iii) The research interests of M. Delbruck, S. Luria, and what came to be called the phage group evolved in complete separation from the bacteriological investigations that made transformation of central concern in Avery's laboratories. Thus although the phage group was certainly aware of the DNA discovery, they did not pursue it: their interests were elsewhere.

The evidence that the phage group knew of Avery's results is fascinating. In the early forties, Delbruck was at Vanderbilt; so was Roy Avery, the recipient of Oswald's May 1943 letter which included the uncharacteristically speculative suggestion that the transforming principle might be the genetic material. According to Delbruck, he met Roy Avery "...the day he received the letter and he told me about it; and I read it then."[62] Luria was introduced to transformation by Dobzhansky's widely read book Genetics and the Origin of Species (second edition, 1941). Since Luria was in New York when he read that book, he immediately went to the Rockefeller to talk to Avery about it. As Luria recalls it: "This was sometime in the spring of 1943, or earlier, before he published. And Avery told me the whole story, how it seemed to be nucleic acids, and so on."[63] There can be no doubt; at roughly the same time, Delbruck and Luria, from the same unimpeachable source, learned of the DNA discovery.

Nevertheless, there is equally strong evidence that the phage group was unwilling to investigate the phenomenon of transformation. Delbruck's reaction to the Avery paper was less than favorable. Recently recalling the debates in the forties about whether DNA had sufficient chemical articulation to be able to carry the specificity required of the genetic material, Delbruck said:

> I distinctly remember wading here [Cold Spring Harbor] at low tide with Rollin Hotchkiss, every so often, and he plugging for the idea that DNA might contain enough specificity...And even after people began to believe it might be DNA, that wasn't really so fundamentally a new

> story, because it just meant that genetic specificity was carried by some goddamn other macromolecule, instead of proteins. 64

This is a deeply revealing statement. It shows that Delbruck considered chemical considerations to be alien to genetics.

For Delbruck, whether genes were made of this or that macromolecule was tangential to what was behind his very entrance into biology: understanding the process of genetic reproduction. This being the goal, viral replication was an attractive system to work with; because it presented the phenomenon of genetic reproduction in what seemed to be it's purest form. Delbruck's own inclination was to reach for understanding by combining physical and genetical considerations: bypassing chemistry.[65] His missionary zeal in promoting this particular research program gave to the phage group, as a whole, an unconcern with chemical considerations. In retrospect, Luria recognized this as a deficiency in the way the phage group was thinking. Luria recalls:

> I had great admiration for him [Avery]. But he was certainly working in something that seemed very different -- but then, I must admit, there was probably a weakness in much of our thinking. ...we were blocked in biochemical thinking. People like Delbruck and myself, not only were not thinking biochemically, but we were somehow -- and probably partly unconsciously -- reacting negatively to biochemistry. And biochemists. As such. As a result, for example, I don't think we attached great importance to whether the gene was protein or nucleic acid. The important thing for us was that the gene had the characteristics it <u>had</u> to have. And that's why Watson and Crick were so tremendously important to us, as <u>genetic</u> thinkers. Because their structure had embedded in it -- one saw immediately -- the properties of the gene. 66

There was a possiblity that the phage group masked from itself. It was the possiblity that the functional nature of the gene might be manifest in its chemical structure.

I have yet to mention one result of the phage group which will seem especially relevant to any discussion of the reception of Avery's work by that research group. The announcement by Hershey and Chase in 1952 that "...sulfur-containing protein has no function in phage multiplication, and that DNA has some function" is touted in textbooks and conversations as being the crucial piece of evidence that converted microbial geneticists to the view that genes were not made of protein but of DNA.[67] Since the evidence of 1952 in favor of the genetic role of (phage) DNA was less clear than the evidence offered by Avery and his descendents at the Rockefeller, if the 1952 evidence was the decisive factor, there is reason to believe that this effect of Hershey and Chase was not based solely on the quality of their evidence. The phage data was much less decisive in ruling out the possibility of protein contamination. Given the different quality of the 1944 and the 1952 evidence, the different receptions of the two experiments deserves explanation.

I am most tempted by sociological explanations. The commitment of the phage group to phage (and to itself) resulted in its members allowing themselves to be convinced by phage data that DNA = gene, even when better pneumococcal evidence had not been able to convince them of the same equation. The fact that such an explanation gives a central role to an emotional commitment to certain kinds of evidence may be sufficient reason to look for another explanation. Olby, for example, denies that "...it can be said that the phage group as a whole jumped on the DNA trail in 1952."[68] McCarty's view is that that although the phage group did jump on the DNA trail after Hershey and Chase's results, this would never have happened without the pneumococcal evidence.[69]

For my purposes, what is significant about the phage groups ignoring of Avery's work is this. They ignored it because they were unconcerned with precisely those chemical questions whose answers opened up the field we now know as molecular genetics. From this point of view Hershey and Chase are not as significant for their results, as they are for their questions. Indeed from this point of view Luria's suggestion -- before Hershey and Chase -- that phage results indicated that protein was the genetic material is just as significant.[70]

(iv) Almost immediately after the 1944 publication, E. Chargaff picked up those biochemical questions which make his important work on the chemistry of nucleic acids the bridge between Avery at the Rockefeller Institute and Watson and Crick at the Cavendish Laboratory.

None of the three ways of responding to Avery's work which I have already discussed involved chemists. Chargaff was a chemist. In 1947, he wrote:

> If, as we may take for granted on the basis of the very convincing work of Avery and his associates, certain bacterial nucleic acids of the desoxyribose type are endowed with specific biological activity, a quest for the chemical or physical causes of these specificities appears appropriate, though it may remain completely speculative for the time being. 71

Chargaff's acknowledgment that Avery had proved that DNA was the genetic material posed his first question. If genes were DNA then the chemical composition of DNA ought to vary from species to species. He set out to determine the molar proportions of the purine and pyrimidine components of DNA samples derived from several species.[72] As he had suspected, there were species specific differences between the molar proportions of purines and pyrimidines in DNA derived from these three sources. Chargaff's results revealed first that DNA was not composed of equal molar amounts of all the nucleotides and second that the unequal amounts varied from species to species. In a 1950 review of this work, Chargaff claimed that these results "...serve to disprove the tetranucleotide hypothesis."[73]

It is hard to underestimate the importance of this result. It constituted a frontal assault on the main support of all those who had doubted that DNA could possibly serve as the genetic material. DNA was revealed not to be a repetitive, uniform substance, and it was revealed to be species specific. However these results were not the most significant ones to come out of Chargaff's laboratory in the late 1940's and early 1950's.

Chargaff and his co-workers noticed that their evidence for the species specificity of the chemical composition of DNA exhibited some surprising regularities. The total molar quantity of both purines (adenine and guanine) was always roughly the same as the total molar quantity of both pyrimidines (thymine and cytosine). Furthermore the molar amount of adenine was roughly the same as that of thymine; and that of guanine was roughly the same as that of cystosine. These three relationships are often presented pictorially thus:

$$A + G = T + C$$

$$A = T$$

$$G = C$$

As is now well known, these three molar equalities were finally given a chemical explanation by the Watson-Crick structure of DNA which reveals that A is bonded to T and G to C. The equimolarities discovered by Chargaff thus proved to be at the heart both of the chemical structure of DNA's famous double helix and of the mechanism of DNA transcription and replication. Chargaff's manner of pursuing the Avery results is the one highlighted by hindsight.

Summary. This last section presented a brief sketch of four ways Avery's "Studies" were acknowledged. (i) The team at the Rockefeller Institute, from which I have isolated Hotchkiss, continued to address Avery's own questions: is transformation a general phenomenon, and is there any protein contamination in the DNA function? (ii) Lederberg saw the Avery result as suggesting Demanding proof that the existence of Mendelian Genetics at the bacterial level. (iii) Delbruck, Luria, and the Phage group saw the Avery result as old-fashioned bacteriological research with perhaps the significance of revealing that genes were made of this rather than that macromolecule. (iv) Chargaff saw Avery's results not as bound to Avery's own conservative questions, nor as linked to Mendelian genetics, nor as a minor investigation of a rather odd bacteriological phenomenon: in Chargaff's eyes Avery's results gave chemistry wings.

(E) Kuhnian Analysis: Revolutionaries or Puzzle-Solvers?

I am not about to launch a full scale interpretation and defense of what Kuhn would call a scientific revolution; because there is much in Kuhn's picture of discontinuous scientific change that cannot be adequately defended. For my purposes, we may say that a Kuhnian revolution consists in the change of paradigm; and I will restrict the meaning of that notoriously abused word to the definition given by Kuhn in the preface to The Structure of Scientific Revolutions: "...paradigms. These I take to be universally recognized scientific achievements that for a time provide model problems and solutions to a community of practitioners."[74] Paradigms -- concrete scientific achievements -- are paradigmatic in a number of ways, two of which are especially relevant here. They provide examples of what a scientific problem is like, and they provide examples of what a solution to that kind of problem ought to look like. Paradigms function in this way by providing examples of judgments which young scientists learn directly. When paradigms change, it is clear that this may or may not cause a change in the collective judgment that this is what a fruitful scientific question looks like and that is what an acceptable scientific answer looks like. Nevertheless, such changes are not, on Kuhn's account, impossible: "...when paradigms change, there are usually significant shifts in the criteria determining the legitimacy both of problems and proposed solutions."[75]

Thus in order to determine whether the Avery result was revolutionary, it will be sufficient to determine whether the three "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumoccal Types" published in 1944 and 1946 guided future research by being used as paradigmatic examples of a new style of asking and answering scientific questions. However, one consequence of Kuhn's analysis of paradigm change is that this is no longer a simple question. We must consider each of the four different research traditions into which those "Studies..." were incorporated, and then ask, of each tradition, whether Avery's "Studies..." were functioning as paradigmatic examples of the type of question to ask and of the kind of answer to look for. Of course, we are especially interested in whether genetical investigations were being modeled on Avery's "Studies...;" because the central task of this paper is to decide whether that work was immediately acknowledged as showing how to raise genetic questions biochemically.

I do not doubt that the self-image of Avery, MacLeod and McCarty was that they were first of all puzzle-solvers, not revolutionaries. As the 1944 title makes clear, the puzzle was to determine "...the chemical nature of the substance inducing transformation of pneumococcal types," and it was the peculiar good fortune of this group that the solution to their puzzle had broad biological implications. Furthermore, Hotchkiss and Avery's other successors at the Rockefeller Institute seem to have understood their own research as filling in the details of the picture of transformation, just as Avery had seen himself as filling in the details of Griffith's picture. If there is a paradigm in the neighborhood -- a concrete scientific achievement which provided model problems and solutions for a community of scientists -- it is Griffith's original paper of 1928: not Avery's of 1944. Avery's paper is an elegant solution to a question arising naturally from Griffith's work. It is not, first of all, a beginning; it is an end.

protein vs polys vs

One surprising result of this analysis is that Delbruck's and Hotchkiss' very different investigations can be seen as having acknowledged Avery's work in the same way. Although the former chose to ignore and the latter chose to pursue the questions raised in Avery's "Studies...," their different research activities showed that they both understood Avery's work as the culmination of an investigation of a question originally raised by Griffith in 1928. Hotchkiss continued to care about that question, Delbruck did not. Nevertheless, they both placed that work within the same space of possible questions.

On the other hand, Lederberg and Chargaff seem to have used Avery's papers as the occasion for elaborating their own questions on the basis of Avery's results. Whereas Hotchkiss may be said to have pursued questions raised <u>in</u> Avery's "Studies...," these two scientists may be said to have pursued questions raised <u>by</u> those "Studies...." Rather than refining Avery's solutions to the qestions he raised, and rather than ignoring those questions, they allowed Avery's discovery to direct their inquiries towards questions which -- however much they were causally dependent on Avery's result -- were not raised or addressed by Avery's "Studies...." Neither Lederberg nor Chargaff can be said to have modeled their questions and techniques on the questions and techniques used with such skill by Avery, MacLeod, McCarty, and their descendents. In each case, they raised new questions which they pursued in new ways, and from these two perspectives Avery's work must again seem to be the work of puzzle-solvers, not revolutionaries.

In evaluating the scientific response to Avery's "Studies on the Chemical Nature of the Substance Inducing Transformation of

Pneumococcal Types" we should discriminate two types of favorable response. First there is the response of those who were continuing questions raised in those "Studies...." These scientists may be said to have used the Avery work (and, once removed, Griffith's work) as a paradigm in planning their own investigations. We might say their work is internally related to Avery's; it feeds of the questions Avery addressed and the way he addressed them. Second there is the response of those whose questions take Avery's work for granted and build on it, but do not model their own work on Avery's. Avery's DNA discovery was instrumental in turning the attention of geneticists to bacteria and of chemists to DNA, but the founders of molecular biology did not model their investigations on Avery's. We might say their work is externally related to Avery's "Studies..."; it builds on Avery's results without building on the way Avery arrived at those results.[76]

Some may believe that because Avery's discovery was instrumental in getting the likes of Chargaff and Watson to investigate DNA, it was therefore revolutionary. I am not tempted by this account; because it appears to be the start of an infinite regress; because it looks as though similar reasoning could make George III the revolutionary source of the American Revolution; and because I think it hides from us the true nature of the change in biological practice which occurred in the middle of this century.

Thus in the narrow Kuhnian sense sketched at the start of this section Avery, MacLeod and McCarty were not revolutionaries.

(F) The Invention of Theoretical Genetics: 1953.

If Avery's work was not paradigmatic for molecular biology, what was? My discussion in sections (D) and (E) may seem to have arrived at the point of giving that role to Chargaff. But I will not.

In 1953, Watson and Crick did provide a universally recognized scientific achievement that for a time provided model problems and solutions to a community of practitioners (roughly molecular biologists). Theirs is the <u>revolutionary</u> example of how to ask biologically significant questions at the molecular level. Putting it this way, what is significant is not the chemical structure of DNA, but what they did with it: not their first paper of 1953 but the second which was called, "Genetical Implications of the Structure of Deoxyribonucleic Acid."[77]

This is not the place to launch an investigation of whether my hypothesis that the significant change in biological practice was the invention of theoretical genetics; and I would never claim that no one but Watson and Crick could have seen the genetical implications of the structure they built to account for the Franklin's and Wilkins' pictures. However I am struck by Chargaff's description of what made molecular genetics so new. His ungenerous reaction to the fame of Watson, Crick, and their descendants defines what was revolutionary about their work. Chargaff described that work as part of "... the <u>new science</u> which grew out of the fusion of chemistry, physics, and genetics, i.e., molecular biology...."[78] Recalling a meeting he had with Watson and Crick in May 1952, Chargaff writes:

> What I did not then realize was that we were on the threshold of a new kind of science: a normative biology in which reality only serves to corroborate predictions; and if it fails to do so, it is replaced by another reality... What is currently considered as the structure of deoxyribonucleic acid was established by people who required no recourse to actual DNA preparations....It was clear to me that I was faced with a novelty: enormous ambition and aggressiveness, coupled with an almost complete ignorance of, and a contempt for chemistry, that most real of exact sciences -- a contempt that was later to have a nefarious influence on the development of "molecular biology." 79

I want to emphasize Chargaff's disgust at the unrepentant ignorance of Watson and Crick: "If they had heard before [1952] about the pairing rules [first reported in 1950], they concealed it. But as they did not seem to know much about anything, I was not unduly surprised."[80] Perhaps more than anyone else, Chargaff who was at the threshold of this new science, recognized its differences from what he had thought of as biology and biochemistry. Thinking back on that 1952 meeting with Watson and Crick he comments: "I am sure that, had I had more contact with, for instance, theoretical physicists, my astonishment would have been less great."[81] It is striking that the explosion of molecular biology after 1953 drew physicists such as Gamow into biology.

My hypothesis that Watson and Crick's papers of 1953 were revolutionary because they provided paradigm examples of a <u>new type of question</u>, and a <u>new way of finding answers</u> was corroborated by Delbruck in a conversation with Judson, who reports: Pauling

> ...I asked [whether] what the Watson-Crick structure did was define the problems next to be solved? Delbruck said, slowly, "Yes. Yes. Yes -- it gave a marvelous fixed point from which to start on both these problems. Replication and Readout. Marvelous in that it was so concrete...I mean that it gave the hope that the whole solution will be possible in

terms of concrete three-dimensional chemistry. Stereochemistry. Enzyme chemistry. Which before was not clear. 82

(G) Conclusion

Having completed my defense of the claim that Avery, MacLeod and McCarty were not revolutionaries, and an outline of why Watson and Crick might have played that role, I am struck by the uncontentiousness of these claims and set to wondering why there have been such excited exchanges concerning the acceptance or recognition of Avery's DNA discovery.

One explanation is that those exchanges have focussed on whether Avery, MacLeod and McCarty on the one hand or biologists in general on the other, recognized the full significance of the 1944 results. In this paper I have sided with those, such as Lederberg, who assert that the full significance of this discovery was recognized both by its discoverers and by most biologists. Nevertheless resolving this question leaves behind the issue I have been addressing in this paper: explaining the delay between the recognition that DNA = gene and the scientific investigation of that equation.

G.S. Stent has explained this delay with reference to what he calls the <u>prematurity</u> of Avery's work. Stent writes that "a discovery is premature if its implications cannot be connected by a series of simple logical steps to cannonical or generally accepted knowledge."[83] I have a suspicion that every surprising result would count as premature by this criterion. Perhaps Stent would say that only those surprising results which are not fruitfully pursued soon after their publication are premature, but this threatens to become the tautology that some surprising results are not fruitfully pursued because they are not fruitfully pursued.

Even if Stent's account of prematurity can be saved from tautology, his particular account of Avery's work is inadequate. He claims that the longevity of the tetranucleotide hypothesis made it impossible for geneticists to think of DNA as the carrier of genetic specificity. If this means that geneticists could not believe that transformation consisted in the transfer of genetic material from one pneumococcus to another, then Stent's claim is false. We have seen that a number of geneticists believed just that. This is not news, the attack on Stent by those who have investigated these matters has been virtually unanimous, but my investigation suggests that in a different sense Avery's DNA discovery was indeed ahead of its time.

Stent's emphasis is on the "...conceptual difficulty of assigning the genetic role to DNA...."[84] However the problem was not <u>conceptual</u>; it was <u>PRACTICAL</u>. The elaboration of the 1944 paper in 1946 by McCarty and Avery, and later by Hotchkiss, successfully answered the conceptual point. But they did not address the practical problem of what to do next. For those many scientists who were able to accept that genes were made of DNA, it was still not clear what to do. Since the chemical structure of DNA wears its biological function on its sleeve, we cannot resist thinking that the obvious thing would have been to try to determine that chemical structure. But as Crick observes, this is a distortion of hindsight: "Nowadays everyone swears they had powers of prediction, and knew from the outset [1944?] that the DNA structure would turn out to be significant. But this is arrant nonsense. Nobody knew.... This part is luck."[85] Avery's work was ahead of its time, not in being unable to be conceived genetically, but in being unable to be investigated genetically.

I am struck by the fact that, even as he argues that the DNA discovery was not conceptually premature in Stent's sense, McCarty appears to concede that it was premature in a practical sense. McCarty: "I would argue that the discovery was not 'premature' but rather required further biological, chemical, and structural development before it could be manipulated by the geneticists."[86] Avery's work was not conceptually ahead of its time, but since we now know of the explosion of biochemical genetics after 1953, it cannot but seem to have been practically ahead of its time.[87]

Since I have discussed various ways Avery's work was pursued in the forties, my view cannot be that it was impossible to pursue Avery's work in 1944. My view is rather that what hindsight reveals as the most significant way to pursue the DNA discovery was not possible until Watson, Crick, and their descendents provide examples of how to investigate genetic questions at the level of biochemistry. You might say it was not possible until the invention of molecular biology, but that is another story. A story involving, in addition to theoretical innovations, innovations as concrete as the end of World War II, the creation and generous funding of several National Institutes of Health, and the ready availability of the familiar machinery of modern biological laboratories.

What I have attempted to do in this paper is to place Avery, MacLeod, and McCarty's discovery not in the history of scientific awards, but in the history of science. It is beyond dispute that Avery's identification of the chemical nature of the genetic material was a *sine qua non* of the development of molecular genetics. But I have argued that Avery's work did not function as a model for how to pursue molecular genetics, and hence that it was not a revolutionary discovery in Kuhn's sense.[88]

FOOTNOTES

1. The dispute is well represented by:
   H.V. Wyatt, "When does information become knowledge?" Nature vol. 235 (January 14, 1972), pp. 86-89.
   J. Lederberg "Reply to Wyatt" Nature vol. 239 (September 22, 1972), p. 234.
   R. Olby "Avery in Retrospect" Nature vol. 239 (September 29, 1972), pp. 295-296.

2. O.T Avery, C.M. MacLeod, and M. McCarty, "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types. Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated From Pneumococcus Type III" J. Exp. Med. v. 79 n. 2 (1944), pp. 137-158.

3. F. Griffith, "The Significance of Pneumococcal Types" J. Hyg. v. 27 n. 2 (1928), pp. 113-159.

4. Ibid., p. 130.

5. Ibid., p. 130.

6. R.J. Dubos, The Professor, The Institute, and DNA (New York: The Rockefeller University Press, 1976), pp. 130-131.

7. Griffith, "The Significance...", pp. 156-157. In a conversation with H. F. Judson, S. Luria "...damned bacteriology as the last stronghold of Lamarckism." See: H.F. Judson, The Eighth Day of Creation (New York: Simon and Schuster, 1979), p. 55.

8. R.D. Hotchkiss, "Oswald T. Avery 1877-1955," Genetics v. 51 (January 1965), p. 4.

9. Dubos, The Professor..., pp. 137-141. Also see: M. McCarty, The Transforming Principle: Discovering That Genes Are Made of DNA (New York: W. W. Norton & Company, 1985), pp. 79-100.

10. J.L. Alloway, "The Transformation In Vitro of R Pneumococci Into S Forms of Different Specific Types by the use of Filtered Pneumococcus Extract" J. Exp. Med. v. 55 (1932), pp. 91-99.

11. For an account of the possible cause of the hiatus, see McCarty, The Transforming Principle, pp. 96-100.

12. O.T. Avery, Letter to his Brother Roy, May 26, 1943. A long extract from this letter is printed in Dubos, The Professor..., pp. 217-220; the passage cited above is on p. 218. A shorter extract, which in a few trivial

matters does not agree with that of Dubos, was printed in McCarty, The Transforming Principle, pp. 157-159. I have not checked the original.

13. J.L. Alloway, (1932), cited in Dubos, The Professor..., p. 138.

14. McCarty, The Transforming Principle, p. 106.

15. I have not summarized the general properties of X, nor the ratio of nitrogen to phosphorus in X, nor the behavior of X in electrical and centrifugal fields.

16. Avery, MacLeod and McCarty, "Studies..." I (1944), p. 150.

17. *Ibid.*, pp. 144-145.

18. *Ibid.*, pp. 145-150.

19. *Ibid.*, p. 151.

20. Avery, MacLeod, and McCarty, "Studies..." I (1944), p. 155.

21. Avery, MacLeod, and McCarty, "Studies..." I(1944), p. 156.

22. Avery, MacLeod, and McCarty, "Studies..." I (1944), p. 153.

23. W.M. Stanley, "The 'Undiscovered' Discovery" Arch. Env. Health v. 21 (1970), p. 259.

24. McCarty, The Transforming Principle, p. 164.

25. Avery's letter to his brother Roy, May 1943, published in Dubos, The Professor..., p. 220.

26. C. MacLeod in conversation with R. Olby September 1968 cited in R. Olby, The Path to the Double Helix (Seattle: University of Washington Press, 1974), p. 188.

27. Dubos, The Professor..., p. 159, also see p. 95.

28. M. McCarty and O.T. Avery, "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types. II. Effect of Desoxyribonuclease on the Biological Activity of the Transforming Substance" J. Exp. Med. v. 83 (1946a), pp. 89-96.
M. McCarty and O.T. Avery, "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types. III. An Improved Method for the Isolation of the Transforming Substance and its Application to Pneumococcus Types II, III, and VI" J.

Exp. Med. v. 83 (1946b), pp. 97-104.

29. McCarty and Avery, "Studies..." II (1946a), p. 90.

30. Ibid., p. 94.

31. Ibid., p. 94.

32. McCarty, The Transforming Principle, p. 190-1.

33. Ibid., p. 216.

34. A.E. Mirsky, published contribution to discussion of Boivin's paper Cold Spring Harbor Symp. Quant. Biol. v. 12, (1947), p. 16.

35. A.E. Mirsky, "Some Aspects of the Cell Nucleus" in Genetics in the Twentieth Century: Essays on the Progress of Genetics During its First Fifty Years, ed. L.C. Dunn (New York: 1951), p. 132.

36. H.J. Muller, "The Gene. Pilgrim Trust Lecture" Proc. Roy. Soc. London B. v. 134 (1947), p. 23n, footnote added in January 1946 to a lecture originally delivered November 1945. By this time, Jan. 1946, Mirsky would have known of McCarty and Avery's DNase evidence; see: McCarty, The Transforming Principle, p. 183.

37. M. Delbruck, in conversation with Judson in Judson, The Eighth Day..., p. 59.

38. Avery's letter to his brother Roy, May 26, 1943, published in Dubos, The Professor..., p. 219-220.

39. McCarty, The Transforming Principle, p. 194.

40. Avery, MacLeod, and McCarty, "Studies..." I (1944), p. 155; Dobzhansky's interpretation was published in the 1941 edition of his Genetics and the Origin of the Species, p. 47.

41. Avery, MacLeod, and McCarty, "Studies..." I (1944), p. 154.

42. Dubos, The Professor..., p. 155.

43. McCarty, The Transforming Principle, p. 194.

44. O.T. Avery's letter to Roy Avery in Dubos, The Professor..., p. 219.

45. Muller, "The Gene," p. 23, first read November 1945.

46. Avery, MacLeod, and McCarty, "Studies..." I (1944), p. 137.

47. T. Dobzhansky, cited in Ibid., p. 155.

48. McCarty, and Avery, "Studies..." II (1946a), p. 95.

49. A. Boivin, "Directed Mutation in Colon Bacilli, By an Inducing Principle of Desoxyribonucleic Nature: Its Meaning for the General Biochemistry of Heredity" Cold Spring Harbor Symp. Quant. Biol. v. 12, (1947), p. 12.

50. McCarty, The Transforming Principle, p. 218.

51. S. Wright, "Physiological Aspects of Genetics" Ann. Rev. Physiol. v. 7 (1945), 83.

52. See: H.V. Wyatt, "When Does Information Become Knowledge?" Nature v. 235 (Jan. 14, 1972), pp.86-89.

53. See: J. Lederberg, "Reply to H.V. Wyatt" Nature v. 239 (Sept. 22, 1972), p. 234.

54. R. Olby, "Avery in Retrospect" Nature v. 239 (Sept. 29, 1972), p. 295.

55. Ibid., p. 296.

56. The biographical information in this paragraph and the next is derived from McCarty, The Transforming Principle, Chapter XI.

57. The idea of extending transformation to other markers first occured to Hotchkiss around 1943. (See: R.D. Hotchkiss, "Gene, Transforming Principle, and DNA" in Phage and the Origins of Molecular Biology, J. Cairns, G.S. Stent, and J.D. Watson, eds., (Cold Spring Harbor, New York, 1966), p. 187.) One of his successes is marked in his "Transfer of Penicillan Resistance in Pneumococci by the Desoxyribonucleate Derived from Resistant Cultures" Cold Spring Harbor Symp. Quant. Biol. v. 16 (1951), pp. 457-461.

58. Hotchkiss, "Gene, Transforming Principle, and DNA," p. 189.

59. J. Lederberg, "Francis J. Ryan" in University on the Heights, W. First, ed., (New York: Doubleday, 1969), pp. 106-107.

60. Ibid., pp. 107-108.

61. J. Lederberg, "Gene Recombination and Linked Segregations in Escherichia Coli" Genetics v. 32 (1947), p. 521.

62. M. Delbruck, in conversation with Judson in Judson, The Eighth Day..., p. 59.

63. S. Luria, in conversation with Judson in Ibid., p. 63.

64. Delbruck, in conversation with Judson in Ibid., p. 60.

65. Olby, The Path to the Double Helix, p. 238.

66. Luria, in conversation with Judson in Judson, The Eighth Day..., p. 62-63.

67. A.D. Hershey and M. Chase, "Independent Functions of Viral Protein and Nucleic Acid in the Growth of Bacteriophage" J. Gen. Physiol. v. 36 (1952), p. 54.

68. Olby, The Path to the Double Helix, pp. 319-320. Also see: McCarty, The Transforming Principle, p. 224.

69. McCarty, The Transforming Principle, p. 224.

70. Luria, in conversation with Judson in Ibid., p. 63, my emphasis.

71. E. Chargaff, "Nucleic Acids and Nucleo Proteins of Micro-Organisms" Cold Spring Harbor Symp. Quant. Biol. v. 12 (1947), p. 30.

72. E. Chargaff, Heraclitean Fire (New York: Warner Books, Inc., 1980), p. 92. This book was first published in New York by The Rockefeller University Press in 1978.

73. E. Chargaff, "Chemical Specificity of Nucleic Acids..." Experientia v. 6 (1950) cited in Chargaff, Heraclitean Fire, p. 93.

74. T.S. Kuhn, The Structure of Scientific Revolutions (Chicago: University of Chicago Press, 1970), p. viii. This book was first published in 1962.

75. Ibid., p. 109.

76. This distinction between internal and external dependence on Avery's "Studies..." might heplfully be elaborated on analogy with J.L. Austin's distinction between the illocutionary act performed IN saying something and the perlocutionary act performed BY saying something. See: J.L. Austin, How To Do Things With Words The William James Lectures of 1955. (London: Oxford University Press, 1975), Chapters VIII and IX.

77. J.D. Watson and F.H.C. Crick, "Genetical Implications of the Structure of Deoxyribonucleic Acid," Nature v. 171 (1953), pp. 964-969. (Olby tells us that this paper was written "chiefly by Crick...." Daedalus Fall 1970, p. 963.)

78. Chargaff, <u>Heraclitean Fire</u>, p. 106.

79. <u>Ibid</u>., p. 100.

80. <u>Ibid</u>., p. 102.

81. <u>Ibid</u>., p. 102.

82. Delbruck, in conversation with Judson in Judson, <u>The Eighth Day...</u>, p. 61.

83. G.S. Stent, "Prematurity and Uniqueness in Scientific Discovery" <u>Sci. Am.</u>, v. 227 (December 1972), p. 84.

84. <u>Ibid</u>., p. 86.

85. F.H.C. Crick, in conversation with Anne Sayre in A. Sayre, <u>Rosalind Franklin And DNA</u> (New York: W. W. Norton, 1975), p. 214n.

86. McCarty, <u>The Transforming Principle</u>, p. 227.

87. My interpretation is closest to that of H.V. Wyatt, according to whom "...we may extend Stent's use of 'premature' and my use of 'knowledge and information' if we include a new concept: discovery can be premature if it is not capable of being extended experimentally because of technical reasons. Griffith's discovery of transformation was extended because this was technically feasible, and in the next 10 years steady progress was made by Avery's associates at the Rockefeller Institute. Each step brought Avery nearer to the identity of the transforming principle. It was only when a new paradigm was desirable in 1944 that the story seemed less clear." See: H.V. Wyatt, "Knowledge and Prematurity: The Journey from Transformation to DNA" <u>Persp. Biol. & Med.</u>, v. 18 (Winter 1975), pp. 149-156, esp. pp. 149-150.

For reasons which are not entirely clear to me, McCarty disagrees with Wyatt. Perhaps he is thinking that in 1944 it was not impossible to pursue the DNA work experimentally. This is true. But, as McCarty himself admits, further work was required before the discovery could be "...manipulated by the geneticists." See: McCarty, <u>The Transforming Principle</u>, pp. 227-8.

88. My analysis of the events discussed in this paper was immeasurably improved by conversations with M. McCarty and J. Lederberg. Earlier versions of the present paper profitted from the constructive criticism of M. Grene, A.G. Bearn, and G.L. Vankin. Detailed suggestions of P. Choppin proved especially helpful in preparing this final version.